

## LETTERS TO THE EDITOR

### Association of Gene Polymorphisms With Coronary Artery Disease in Low- or High-Risk Subjects Defined by Conventional Risk Factors

We read with interest the careful study by Hirashiki et al. (1), who, upon examining 37 single nucleotide polymorphisms (SNPs) in 31 candidate genes, reported a significant association of coronary artery disease (CAD) with SNPs in the apolipoprotein E, connexin-37, stromelysin-1, and endothelial nitric oxide synthase (eNOS) genes. A polymorphism in the promoter ( $T^{-786}C$ ) of the latter gene was recently shown to bear functional consequences (2) because the C allele creates a binding site for a replication protein A-1 that acts as a repressor of gene transcription (3). Accordingly, essential hypertensive patients carrying this allele exhibited a blunted forearm vasodilation in response to acetylcholine (4). This allele was shown to be significantly associated with coronary vasospasm and myocardial infarction (MI) in Japanese patients (2,5) and with multivessel CAD in consecutive Caucasian patients undergoing coronary angiography because of suspected CAD (6). Thus, the results of Hirashiki et al. (1) in the Japanese patient population appear to be fully confirmatory of earlier reports (5), including our study of Caucasians (6), that were overlooked.

Based on the presence or absence of hypertension, hypercholesterolemia, and diabetes mellitus, subjects in the Hirashiki et al. study (1) were divided into a high- and a low-risk group, respectively, yet about 74% of their CAD patients had myocardial infarction (MI) and therefore cannot be considered at low risk. Indeed, according to the Third Report of the National Cholesterol Education Program Expert Panel (Adult Treatment Panel III) (7), these individuals would represent high-risk patients (e.g., subjects with a  $>20\%$  risk of cardiovascular events in the next 10 years). Moreover, as the investigators correctly pointed out, their population comprises MI survivors. Thus, it remains unclear whether the SNPs that showed a significant association with CAD in a population that mainly comprised high-risk MI survivors represented markers of survival or of predisposition to CAD (e.g., protective or nefarious genetic variants). In our consecutive patients, a minority (45.6%) had a history of MI (6). Accordingly, although the mortality for MI might be higher in Western countries than in Japan, the majority of our patient population did not comprise MI survivors.

The significant association of the  $T^{-786}C$  allele with CAD was confirmed by Hirashiki et al. also in their high-risk men; however, the researchers did not comment on this important finding. Of interest, we tested the hypothesis that the genetic predisposition to generate less nitric oxide (NO) was more detrimental under conditions with blunted NO availability, such as age older than 60 years, hypercholesterolemia, cigarette smoking, and low high density lipoprotein (HDL) cholesterol. With such analysis we found not only a significant association of multivessel CAD with the  $T^{-786}C$  allele but also, more importantly, a marked increase in the relative risk of multivessel CAD from 1.7 (95% confidence interval: 1.11 to 2.55,  $p = 0.014$ ) in the overall population to 3.61 (1.63 to 8.0,  $p = 0.002$ ) when at least four such conditions coexisted. Thus, one of the SNPs that was found to be associated with CAD by Hirashiki et al. (1) does not seem to increase the risk

of CAD independently of other risk factors. Instead, it appears to deeply interact with environmental factors that blunt NO availability.

It must be kept in mind that association studies are peculiarly prone to false positive results, particularly when controls are not randomly selected, as in the study by Hirashiki et al. (1). Based on dividing the patients into low- and high-risk groups, the investigators pinpointed SNPs that were significantly associated with CAD in low- and high-risk groups of men and women. As the investigators correctly acknowledged, the large number of comparisons that were made opened up the possibility of chance findings; furthermore, no information on power of the subgroup analyses was furnished. Nonetheless, the independent replication by Hirashiki et al. in a genetically unrelated population of our report of an association of the  $T^{-786}C$  allele with multivessel CAD lends strong support to our conclusion that this SNP is an important genetic factor predisposing to the development of multivessel CAD.

**Gian Paolo Rossi, MD, FACC, FAHA**

DMCS—Clinica Medica 4  
University Hospital  
via Giustiniani, 2  
35126 Padova  
Italy  
E-mail: gianpaolo.rossi@unipd.it

**Giuseppe Maiolino, MD**

Clinical Cardiology  
University of Padua

doi:10.1016/j.jacc.2004.04.005

## REFERENCES

- Hirashiki A, Yamada Y, Murase Y, et al. Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. *J Am Coll Cardiol* 2003;42:1429–37.
- Nakayama M, Yasue H, Yoshimura M, et al.  $T^{-786}C$  mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation* 1999;99:2864–70.
- Miyamoto Y, Saito Y, Nakayama M, et al. Replication protein A1 reduces transcription of the endothelial nitric oxide synthase gene containing a  $-786T \rightarrow C$  mutation associated with coronary spastic angina. *Hum Mol Genet* 2000;9:2629–37.
- Rossi GP, Taddei S, Virdis A, et al. The  $T^{-786}C$  and Glu298Asp polymorphisms of the endothelial nitric oxide synthase gene affect the forearm blood flow responses of Caucasian hypertensive patients. *J Am Coll Cardiol* 2003;41:938–45.
- Nakayama M, Yasue H, Yoshimura M, et al.  $T^{-786}C$  mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with myocardial infarction, especially without coronary organic stenosis. *Am J Cardiol* 2000;86:628–34.
- Rossi GP, Cesari M, Zanchetta M, et al. The  $T^{-786}C$  endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA study. *J Am Coll Cardiol* 2003;41:930–7.
- Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.

## REPLY

We thank Drs. Rossi and Maiolino for their comments regarding our paper (1). Beyond 2020, it is likely that genetic identification